

CLAIMS

What is claimed is:

1. An isolated, non-canonical zinc finger binding protein comprising one or more
5 non-canonical zinc finger components that bind to a target sequence.

2. The isolated zinc finger binding protein of claim 1, wherein the target sequence
is a nucleic acid sequence.

10 3. The isolated zinc finger binding protein of claim 1, wherein the target sequence
is an amino acid sequence.

15 4. The isolated zinc finger binding protein of claim 2, wherein the target sequence
is DNA.

5. The isolated zinc finger binding protein of claim 2, wherein the target sequence
is RNA.

20 6. The isolated zinc finger binding protein of claim 1, wherein the amino acid
sequence of one or more of the zinc finger components is selected from the group consisting
of: X₃-**B**-X₂₋₄-Cys-X₁₂-His-X₁₋₇-His-X₄; X₃-Cys-X₂₋₄-**B**-X₁₂-His- X₁₋₇-His-X₄; X₃-Cys-X₂₋₄-
Cys-X₁₂-**Z**-X₁₋₇-His-X₄; X₃-Cys-X₂₋₄-Cys-X₁₂-His-X₁₋₇-**Z**-X₄; X₃-**B**-X₂₋₄-**B**-X₁₂-His-X₁₋₇-His-
X₄; X₃-**B**-X₂₋₄-Cys-X₁₂-**Z**-X₁₋₇-His-X₄; X₃-**B**-X₂₋₄-Cys-X₁₂-His-X₁₋₇-**Z**-X₄; X₃-Cys-X₂₋₄-**B**-
X₁₂-**Z**-X₁₋₇-His-X₄; X₃-Cys-X₂₋₄-**B**-X₁₂-His-X₁₋₇-**Z**-X₄; X₃-Cys-X₂₋₄-Cys-X₁₂-**Z**-X₁₋₇-**Z**-X₄;
25 X₃-Cys-X₂₋₄-**B**-X₁₂-**Z**-X₁₋₇-**Z**-X₄; X₃-**B**-X₂₋₄-Cys-X₁₂-**Z**-X₁₋₇-**Z**-X₄; X₃-**B**-X₂₋₄-**B**-X₁₂-His-X₁₋₇-
Z-X₄; X₃-**B**-X₂₋₄-**B**-X₁₂-**Z**-X₁₋₇-His-X₄; and X₃-**B**-X₂₋₄-**B**-X₁₂-**Z**-X₁₋₇-**Z**-X₄, wherein X is any
amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

30 7. The isolated zinc finger binding protein of claim 6, wherein the zinc finger
component comprises the sequence X₃-**B**-X₂₋₄-Cys-X₁₂-His-X₁₋₇-His-X₄, wherein X is any
amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

8. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence X_3 -Cys- X_{2-4} -**B**- X_{12} -His- X_{1-7} -His- X_4 , wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

5

9. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence X_3 -Cys- X_{2-4} -Cys- X_{12} -**Z**- X_{1-7} -His- X_4 , wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

10

10. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence X_3 -Cys- X_{2-4} -Cys- X_{12} -His- X_{1-7} -**Z**- X_4 , wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

15

11. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence X_3 -**B**- X_{2-4} -**B**- X_{12} -His- X_{1-7} -His- X_4 , wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

20

12. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence X_3 -**B**- X_{2-4} -Cys- X_{12} -**Z**- X_{1-7} -His- X_4 , wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

25

13. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence X_3 -**B**- X_{2-4} -Cys- X_{12} -His- X_{1-7} -**Z**- X_4 , wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

14. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence X_3 -Cys- X_{2-4} -**B**- X_{12} -**Z**- X_{1-7} -His- X_4 , wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

15. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence $X_3\text{-Cys-}X_{2-4}\text{-}\mathbf{B}\text{-}X_{12}\text{-His-}X_{1-7}\text{-}\mathbf{Z}\text{-}X_4$, wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

5 16. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence $X_3\text{-Cys-}X_{2-4}\text{-Cys-}X_{12}\text{-}\mathbf{Z}\text{-}X_{1-7}\text{-}\mathbf{Z}\text{-}X_4$, wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

10 17. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence $X_3\text{-Cys-}X_{2-4}\text{-}\mathbf{B}\text{-}X_{12}\text{-}\mathbf{Z}\text{-}X_{1-7}\text{-}\mathbf{Z}\text{-}X_4$, wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

15 18. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence $X_3\text{-}\mathbf{B}\text{-}X_{2-4}\text{-Cys-}X_{12}\text{-}\mathbf{Z}\text{-}X_{1-7}\text{-}\mathbf{Z}\text{-}X_4$, wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

20 19. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence $X_3\text{-}\mathbf{B}\text{-}X_{2-4}\text{-}\mathbf{B}\text{-}X_{12}\text{-His-}X_{1-7}\text{-}\mathbf{Z}\text{-}X_4$, wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

25 20. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence $X_3\text{-}\mathbf{B}\text{-}X_{2-4}\text{-}\mathbf{B}\text{-}X_{12}\text{-}\mathbf{Z}\text{-}X_{1-7}\text{-His-}X_4$, wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

30 21. The isolated zinc finger binding protein of claim 6, wherein the zinc finger component comprises the sequence $X_3\text{-}\mathbf{B}\text{-}X_{2-4}\text{-}\mathbf{B}\text{-}X_{12}\text{-}\mathbf{Z}\text{-}X_{1-7}\text{-}\mathbf{Z}\text{-}X_4$, wherein X is any amino acid, B is any amino acid except cysteine and Z is any amino acid except histidine.

22. The isolated zinc finger binding protein of claim 1, wherein the target sequence is in a plant cell.

23. The isolated zinc finger binding protein of claim 1, wherein the target sequence is in an animal cell.

24. The isolated zinc finger binding protein of claim 23, wherein the target
5 sequence is in a human cell.

25. The isolated zinc finger binding protein of claim 1, wherein the target sequence is a promoter sequence.

26. The isolated zinc finger binding protein of claim 1, comprising three zinc
10 finger components.

27. The isolated zinc finger binding protein of claim 1, wherein the target sequence
15 comprises about 9 to about 14 contiguous base pairs.

28. The isolated zinc finger binding protein of claim 26, wherein the third finger
component comprises a non-canonical zinc finger component

29. The isolated zinc finger binding protein of claim 1, comprising a modified
20 plant ZFP backbone.

30. An isolated polynucleotide encoding a zinc-finger binding protein according to
claim 1.

31. An expression vector comprising the polynucleotide of claim 30.
25

32. A host cell comprising the polynucleotide of claim 30.

33. A fusion polypeptide comprising: (a) an isolated zinc finger binding protein
30 according to claim 1 and (b) at least one functional domain.

34. The fusion polypeptide of claim 33, wherein the functional domain is a repressive domain.

35. The fusion polypeptide of claim 34, wherein the repressive domain is selected from the group consisting of KRAB, MBD-2B, v-ErbA, MBD3, TR and members of the DNMT family.

36. The fusion polypeptide of claim 35, wherein the functional domain is an activation domain.

37. The fusion polypeptide of claim 36, wherein the activation domain is selected from the group consisting of VP16, p65 subunit of NF-kappa B, and VP64.

38. The fusion polypeptide of claim 37, wherein the functional domain is selected from the group consisting of an insulator domain, a chromatin-remodeling protein or a methyl-binding domain.

39. An isolated polynucleotide encoding the fusion polypeptide of claim 33.

40. An expression vector comprising the polynucleotide of claim 39.

41. A host cell comprising the polynucleotide of claim 39.

42. A method of modulating expression of a gene, the method comprising the step of contacting a region of DNA with a fusion molecule according to claim 33.

43. The method of claim 42, wherein the zinc finger binding protein of the fusion molecule binds to a target site in a gene encoding a product selected from the group consisting of vascular endothelial growth factor, erythropoietin, androgen receptor, PPAR- γ 2, p16, p53, pRb, dystrophin and e-cadherin.

44. The method of claim 42, wherein the functional domain comprises a repressive domain.

45. The method of claim 44, wherein the repressive domain is selected from the group consisting of KRAB, MBD-2B, v-ErbA, MBD3, TR and members of the DNMT family.

46. The method of claim 42, wherein the functional domain comprises an activation domain.

47. The method of claim 46, wherein the activation domain is selected from the group consisting of VP16, p65 subunit of NF-kappa B, and VP64.

48. The method of claim 42, wherein the functional domain is selected from the group consisting of an insulator domain, a chromatin-remodeling protein or a methyl-binding domain.

49. The method of claim 42, wherein the gene is in a plant cell.

50. The method of claim 42, wherein the gene is in an animal cell.

51. The method of claim 50, wherein the gene is in a human cell.

52. A pharmaceutical composition comprising a non-canonical zinc finger protein according to claim 1 and a pharmaceutically acceptable excipient.